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(21) International Application Number: PCT/SE88/00183 (22) International Filing Date: 11 April 1988 (11.04.88) (31) Priority Application Number: 8701479-1 (32) Priority Date: 9 April 1987 (09.04.87) (33) Priority Country: SE (71) Applicant (for all designated States except US): CAR-BOMATRIX AB [SE/SE]; Ferievägen 9, S-223 67 Lund (SE). (72) Inventors; and (75) Inventors/Applicants (for US only) : SCHRÖDER, Ulf [SE/SE]; Ferievägen 9, S-223 67 Lund (SE). LAGER, Catharina [SE/SE]; Agardhsgatan 5, S-223 51 Lund (SE). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), LU (European patent), NL (European patent), SE (European patent), US.		Published <i>With international search report.</i> <i>In English translation (filed in Swedish).</i>
(54) Title: A METHOD FOR ENTRAPMENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND THE USE THEREOF (57) Abstract A pharmaceutical formulation is described, which is made of a prefabricated microspher/particle, combined with a pharmaceutically acceptable polymer which delays the release of the entrapped biologically active substance. Also described is the use of the formulation for the biologically active substance.		

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A METHOD FOR ENTRAPMENT OF BIOLOGICALLY ACTIVE SUBSTANCES AND THE USE THEREOF

BACKGROUND:

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This invention relates to a way of produce, use and/or utilize a pharmaceutical formulation for biologically active substances. Such formulations may be used within human and veterinary medicin and in the agricultural areas.

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Examples of this kind of formulations are slow release systems for drugs, targeting of drugs or the use as contrast agents. More directly this invention relates however, to a method for fabrication and use of slow release systems for a biologically active substances using a process which allows entrapment of biologically active substances within

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polymers which are biodegradable and biocompatible. A drug, within the scope of the present invention, is defined in its broadest sense, such as a biologically active substance having effects and/or is used within human and/or veterinary medicin as well as within agricultural areas.

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Within the medical areas one can divide the biologically active substances after there area of use.

Substances for the use within the respiratory tract; cough reducing (e.g. noscapine) or opiates (e.g. ethylmorphine). Mucosmembrane affectors (e.g. ephedrin, terbutalin and theophylline).

25

Heart and bloodvessel agents; glycosides, such as digoxin, kinidin, lidocain, procainamide.

Beta-blocking agents such as alprenolol or metoprolol.

Other groups includes alfa-blocking agents (e.g. phentolamine), beta-stimulators (e.g. bamethan), alkyl nitrates, calcium antagonists (e.g. nifedipin), nicotinic acid derivatives, adrenergics (e.g. adrenalin) sympathetic moderators (e.g. guanetidin) ganglie blockers (e.g. trimetafan), hydrazine derivatives, tiazide derivatives, bensen-sulfonamide derivatives, bumetamide, furoseamide, etacrynic acid, spironolacton.

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Varix treatment (e.g. polidokanol), cholesterol synthesis blockers (e.g. clofibrate).

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Antihistamins (e.g. prometazin, terbutalin) at allergic disorders.

Spasmolytic substances; papeverinderivatives, anticholinergic (e.g. atropin), cholinergic substances (e.g. karbacol).

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Drugs for tumour diseases; vitamins (e.g. B-12 or folic acid), alkylating

- 1 cytostatic drugs (e.g. cyclofosfamid), antibiotics (e.g. daunomycin, bleo-
mycin), mitos blockers (e.g. vinblastin), cisplatinum, nitrosurea
derivatives, estramustin, steroidderivatives, cimetidin, ranitidin.
Chemotherapeutic and antibiotic substances, sulfonamides, peni-
5 cillines, cephalosporins, tetracyclines, aminoglycosides, macrolides,
aminosalicylicacid derivatives, iso-nicotinic acid derivatives, iodine.
Malaria drugs (e.g. clorokin).
Substances against fungus infections (e.g. griseofulvin).
Vitamins.
- 10 Proteins and peptides, digestion enzymes, coagulation factors (e.g.
factor VIII), immunglobulins, vaccines, hormones (e.g. oxytocin),
corticotropins, thyrotropin, growth hormon, anti-diuretic hormon (or
DDAVP), glucocorticoids, mineralcorticoids, androgens, oestrogens,
thyroid hormones, insulin, calcitonin, glucagon, sulfonureids,
enkefalins.
- 15 Immunstimulating substances (e.g. interferons, interleukins).
Psychopharmacological drugs; barbituric acid derivatives, piperindin-
dion derivatives, propandiol derivatives, bensodiazepin derivatives,
fentiazin derivatives, tioxantan derivatives, butyrofenon derivatives,
tricyclic thymoleptic drugs, coffein, antihistaminic substances.
- 20 Antiepileptic drugs (e.g. derivatives of hydantoin).
Muscle relaxation substances (e.g. kinin, curare).
Prostaglandins, nicotinamid.
- 25 Anticholinergic and anesthetic drugs; morfin derivatives, fenylypiperidin
derivatives, diphenylpropylamine derivatives, salicylic acid derivatives,
bensotriazin derivatives, anilides, indol-acetic acid derivatives, fenyly-
acetic acid derivatives, naftyl-acetic acid derivatives, ergotamine
derivatives, serotonin antagonists, clonidin, lidocain derivatives.
- 30 For the person skilled in the art it is obvious that these substances are
not by any means limited to the use within the areas mentioned above,
the substances can be, and are used for other purposes or indications
than the ones described above.
- 35 In agricultural areas substances that are used as herbicides or
stimulators on crop may be used. Also substances that have an affect on
various parasites are included (e.g. pesticides).
- 40 Within the pharmaceutical industry there are at present several
methods described for entrappment of hydrophobic substances.
A hydrophobic substance is characterized by beeing preferentially
solvable in a hydrophobic solvent. This means that the solvent has a
capability of dissolving various fatty substances, such as fatty acids, oils
or the like. Hydrophilic refers to similar solvation capability but for

1 water soluble substances.

However, still there are no acceptable methods for preparation of slow release formulations for hydrophilic substances, due to the technical difficulties, in the manufacturing of such formulations.

5 In principle there are two basic principles to prepare a pharmaceutical formulation for a biologically active substance: entrapment into, or covalent coupling to a matrix. In the case of entrapment you take advantage of the characteristics of the formulation and the biologically active substance have respectively, in order to create association phenomena, resulting in a stable preparation. Of great importance
10 working with formulations within the areas mentioned above, is that the formulation in it self, will not create toxic metabolites. Having a choice with these aspects in mind you are mainly directed towards using materials that are made of endogenous substances or polymerized in biocompatible way.

15 One type of polymers that has attracted large interest during the last years are the use of polymerized hydroxycarboxylic acids. An example of a monomer, which can be used for this type of polymisationer is lactic acid: polymerized into poly-lactic acid (PLA), often polymerized together with glycolic acid. This co-polymer is namned as PLGA (poly-lactic-glycolic acid). Microspheres prepared of PLGA are relatively stable
20 in a physiological enviroment due to the hydrophobic interactions between the hydrophobic PLGA polymer. Another polymer showing the same characteristics is poly-capronic acid.

The great intrest for this type of polymers, in particular in there use in the preparation of microspheres, is reflected in the patent litterature, where a large number of applications and patents dealing with variants
25 of preparations procedures and/or use is described.

The use and the interest for polymers made of PLGA is partly based upon the fact that the monomer is an endogenous substance and partly that the monomers are bonded to each other by ester bonds. These
30 esterbonds are slowly hydrolized in contact with water whereby the original monomer is reformed.

The hydrophobic interactions within the polymer are utilized when the polymer is used as matrix. Since PLGA is a hydrophobic polymer it will
35 adsorb hydrophobic substances.

The hydrophobic interactions are only slowly broken up, preferentially this is seen in connection with hydrolysis of the esterbonds in the polymers, appearing in a hydrophilic enviroment such as a human body.

40 Another type of monomers that can be used for the purpose of this invention, due to their biocompatability, are polymers of oxaloacetate.

1 citrate, isocitrate, oxalosuccinate, ketoglutarate, succinate, fumarate,
malate or a derivative of these.

Another type of acceptable polymer that can be used within the scope
of this invention is a graft polymer between PLA/PLGA and
carbohydrates. This type of polymers are described in Swedish patent
5 application 8601563-3.

Another example of polymers which already have found use within this
area and could be used in connection with this invention are the poly-
anhydrides (e.g. poly-bis(p-carboxyphenoxy)alkane anhydride), poly-
ethylenevinyl acetate, poly-orthoesters, poly-vinyl alcohol, poly-vinyl
10 acetate, poly-vinyl chloride, acrylic polymers, poly-amino acids, poly-
urethane, poly-silanes. Furthermore, there are a number of
combinations and derivatives of these that can be used.

As mentioned above, the main use of PLGA is for hydrophobic low
molecular weight biologically active substances. However, there is a
15 great interest from the pharmaceutical industry to be able to produce
matrix systems for hydrophilic substances. This has however, proven to
be far more difficult than for hydrophobic substances.

The main reason for the difficulty in preparing a hydrophilic matrix
system that can retard a hydrophilic substance in the hydrophilic
20 environment such as a human body, is the following; to be able to
dissolve a hydrophilic substance you need a hydrophilic solvent. Thus,
by preparing a hydrophilic matrix system, this will be rapidly dissolved
in a hydrophilic environment and the entrapped hydrophilic substance
will rapidly be released.

25 Alternatively, if you prepare a hydrophobic matrix system this can be
dissolved in a hydrophobic environment and only very slowly or not at all
in a hydrophilic environment.

By the reasoning above it is obvious that there is a great deal of interest
from the pharmaceutical industry for pharmaceutical formulations for
30 hydrophilic drugs. In particular, it would be advantageous if one could
prepare a formulation for entrapment, since the so formed
formulation would contain the biologically active substance without
having to manipulate the molecular structure of the substance. When,
and if, such a formulation is obtained, documentation regarding
35 toxicity, metabolism and elimination routes of the biologically active
substance would already be at hand due to previous registration and
approval.

As an example it may be worth mentioning that there is a great
interest in preparing microspheres to be used for entrapment of X-
40 ray contrast agents. The main interest is in preparing a microsphere

1 having a diameter of 2 μm which can be injected into the bloodstream.
Such a microsphere should thus be useable as a specific contrast agent
for the reticuloendothelial system (RES). Of particular interest would
be to use the microspheres as a contrast agent for the liver.
Another great interest for the type of formulations, as described in this
5 invention, is if one could prepare a microsphere with entrapped
biologically active substances having a size allowing inhalation, e.g. as a
spray. The use of such a preparation containing e.g. terbutalin or
theophylline, is primarily in the treatment of asthma. Another substance
awoking a new and special interest is nicotinic acid, since it has been
10 shown it has effects at tumour treatment.
Proteins are also incorporated into this group of particularly
interesting substances since this group of substances have high
demands on the preparation methodology in order to protect the three
dimensionell structure.
15 Methods for preparation of known formulations, as discussed in this
invention is mainly based on the following principles: phase eva-
poration, precipitation or spraydrying. As an example of phase
evaporation may be mentioned that PLGA microspheres are primarily
prepared according to this technology (US 4,389,330). Another
20 example of polymers used for the preparation of microspheres are
carbohydrate polymers, e.g. starch, which also are considered to have
fulfilled all the necessary demands in order to be used as a carrier of
biologically active substances.
Precipitation systems (crystallization) is described in PCT/SE83/-
25 00268 where the polymer beeing described is starch. Polymerization
systems is also described for the preparation of starch microspheres
(SE 7407461-8). Also complexes and solutions are described (Swedish
appl. 8501094.0) as useful formulations within this area. However,
starch is to hydrophilic to be used for hydrophilic low molecular
30 weight substances.
However, the invention is not restricted to the use within the
methodologies mentioned above for slow release, the expert in the
field may easily adopt the methodology for the use in other areas where
there is a need for a matrix system according to the present invention.

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DESCRIPTION OF THE INVENTION.

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One possibility for the preparation of a formulation, which is not
described earlier, and which is described in this invention, involves

1 the use of an already existing matrix, formed as a sphere or a particle,
this sphere/particle containing the biologically active substance only,
the sphere or particle subsequently being covered by a surface of a
polymer which does not have the same solubility parameters as the
biologically active substance. In certain cases it might be difficulties in
5 preparing a sphere/particle only using the biologically active substance,
in such a case there is the possibility of mixing the biologically active
substance with a protective colloid. Protective colloids that can be used
are preferentially substances such as carbohydrates, and the
methodology for such a preparation procedure for a sphere/particle is
10 preferentially done according to PCT/SE83/00268.

Of special interest, as shown in this invention, is the possibility of
preparing a sphere/particle of a hydrophilic biologically active
substance, subsequently covered by a surface of a hydrophobic polymer.
A hydrophobic polymer gives, in this special case as a result, that upon
15 suspending the formulation in water, the water will have great
difficulties in penetrating the hydrophobic surface and dissolve the
entrapped hydrophilic substance.

The invention shows that by using this technology, which involves
covering a hydrophilic matrix with a hydrophobic polymer in a process
20 where water is absent, a retardation of the release of the biologically
active substance after suspension of the formulation in a physiological
environment is achieved. The expert in the field can thus easily adopt
and use the described invention for the preparation of formulations
which all shows a slow release effect of the entrapped biologically
25 active substance.

The methodology involves in the first step production of a
sphere/particle of a biologically active substance according to the basic
technology described in PCT/SE83/00268. There is also the possibility
to use a microsphere of crosslinked starch as described in Swedish
30 applications 7407461 or 7900737-3, to which one or more biologically
active substances has been associated. These dried and water free
spheres made of only biologically active substance or a biologically active
substance entrapped, or otherwise associated to a hydrophilic matrix,
is suspended into a hydrophobic solvent having the ability to function
35 as a solvent of a hydrophobic polymer but not to the biologically active
substance. The hydrophobic polymer is dissolved into the hydrophobic
solvent, this solution is added to another solvent, into which said
polymer is insoluble, whereafter the first solvent is removed.

Of importance in the use of the invention for e.g. contrast agents or by
40 inhalation, is that the formulation may be obtained as a suspension in
physiological environment without having the microspheres aggregated.

1 This may be accomplished using known technology, such as
adsorption of detergents on the surface of the microspheres before
these are dried.

The invention is based upon the unexpected result that the hydro-
phobic polymer was shown to cover the surface of the hydrophilic
5 microsphere, resulting in a sphere, to the extent that a high
retardation of the release of the biologically active substance was
obtained after suspension of the formulation in water.

At the process of covering of the hydrophilic microsphere there is also
the possibility to adsorb the hydrophobic polymer using successively
10 higher molecular weight of the polymer. By this process the low
molecular weight polymers will be able to penetrate further into the
hydrophilic structure, whereby is given the possibility to prepare the
formulation with various release rates of the biologically active
substance. In particular, this methodology has shown to be useful by
15 adsorption of the hydrophobic polymer using sonication.

The following example is to be considered as illustrating but not
limiting, since the basic technologies used are well known and can
easily be modified by an expert in the field.

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EXAMPLE 1.

4 gram of polycaprolacton was dissolved in 15 ml of chloroform,
whereafter 1.78 gram of Metrizamid® in the form of microspheres,
25 having a mean diameter of 1 μ m, was suspended. The suspension was
spray dried using a Büchi 190 spray drier. The dried product was
subjected to elementary analysis of the iodine. The product was shown
to contain 12% metrizamid. The product was suspended in water and
the iodine content was determined after 1 hour and was shown to have
30 been reduced to 4.2%. If microspheres of metrizamid (unprocessed)
are suspended in water, the metrizamid is immediately dissolved and
the solubility is, according to the manufacturer, unlimited.

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1 CLAIMS

1.

A method for the preparation of an injectible formulation
characterized by, that one or several biologically active substances
first has been prepared in the form of microspheres/particles,
eventually together with a protective colloid, whereafter this
microsphere/particle is covered by a surface of a polymer that does not
show the solubility characteristics of the biologically active substance.

2.

A formulation according to claim 1, **characterized by**, that the
microsphere/particle, containing only the biologically active substance,
is prepared by crystallization.

3.

A formulation according to claim 1, **characterized by**, that the
protective colloid is a carbohydrate, chosen from the group of starch,
glycogen, pullulan, maltodextrin, glucose or a derivative of these.

4.

A formulation according to claim 1-3, **characterized by**, that the
hydrophobic and biocompatible polymer used at the covering of the
surface of the microsphere/particle is made from hydroxi-carboxylic
acids

5.

A formulation according to claim 1-4, **characterized by**, that the
hydrophobic polymer is from the group polymers containing lactic
acid, glycolic acid, hydroxi-lactonic acid or a graft polymer of these.

6.

A formulation according to claim 1-5, **characterized by**, that the
hydrophobic polymer is from the group of polymers containing
oxaloacetate, citrate, isocitrate, oxalosuccinate, ketoglutarate,
succinate or malate or a derivative of these.

7.

A formulation according to claims 1-6, **characterized by**, that the
matrix preparation is in the form of a capsule, cylinder, microsphere,
complex or a solution.

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The use of the formulation according to claims 1-7 for biologically active substances.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/SE88/00183

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 47/00, 9/22

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System

Classification Symbols

IPC 4

A 61 K 47/00, 9/16, /20, /22, /32, /58

US C1

424:14, 31, 32

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched *

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X,Y,	EP, A, 102 265 (THE STOLLE RESEARCH AND DEVELOPMENT CORPORATION) 7 March 1984 see claims, page 4, lines 11-27 & JP, 59161316 US, 4530840 US, 4542025 CA, 1218306	1,4,5,7,8
X	SE, B, 328 670 (SMITHKLINE & FRENCH LABORATORIES) 21 September 1970 see example	1,2,7,8
X	US, A, 4 479 911 (SANDOZ) 30 October 1984 see claims, col. 2, lines 30-40 and 54-62, col. 5, lines 1-7	1,2,4,5,7,8
X	Patent Abstract of Japan Vol. 9, No. 180 (C-293), abstract of JP 60-48923, published 16 March 1985	1,4,5,7,8
.../...		

* Special categories of cited documents: 10

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

1988-06-09

Date of Mailing of this International Search Report

1988-06-23

International Searching Authority

Swedish Patent Office

Signature of Authorized Officer

Agneta Tannerfeldt

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	SE, B, 413 578 (AS ALFRED BENZON) 9 June 1980 see example 1, page 1, lines 4-8 & NL, 7404134 FR, 2223047 DE, 2414868 GB, 1468172 CH, 585556 CA, 1029658 JP, 50029729	1,7,8
Y	WO, A, 84/00294 (SCHRÖDER ULF) 2 February 1984 see claims & EP, 0113749 AU, 567434 US, 4713249	3
A	EP, A, 26 599 (ELI LILLY AND COMPANY) 8 April 1981	
A	EP, A, 147 335 (LABORATOIRES D'HYGIENE ET DE DIETETIQUE) 3 July 1985	

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim numbers¹ because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out. Specifically:

The claim, which defines the polymer with its solubility property relating to the active component is too vague and unspecified.
The search has therefore been incomplete.

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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